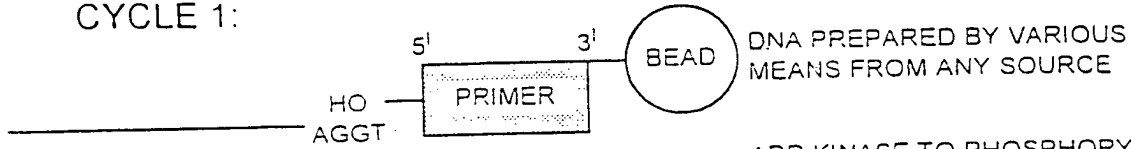
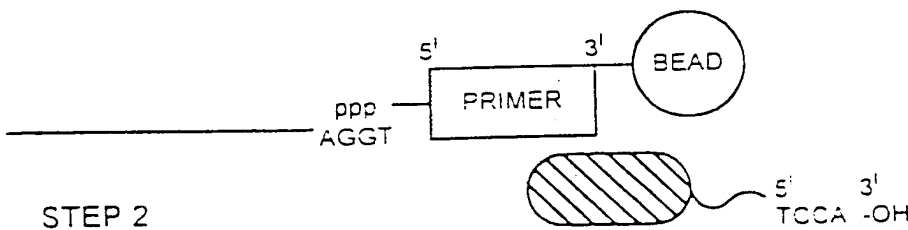


CYCLE 1:



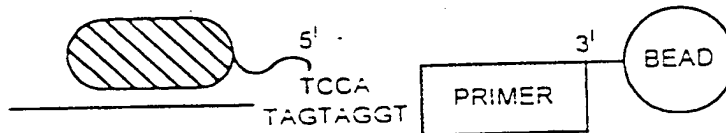
STEP 1

ADD KINASE TO PHOSPHORYLATE 5' TERMINUS OF PRIMER, ACTIVATING THIS TERMINUS FOR EXTENSION BY LIGATION OF 4 MER PROBES.



STEP 2

ADD ALL 256 POSSIBLE 4-MERS IN THE PRESENCE OF A LIGASE. TO EXTEND DOUBLE-STRANDED PRIMER REGION BY 4 BASE PAIRS. THE LIGASE ACTS TO ENSURE THE FIDELITY OF THE EXTENSION.



STEP 3

WASH IN LOW STRINGENCY BUFFER OR RAISE TEMPERATURE OF MEDIUM TO REMOVE ANY UNLIGATED 4-MERS



STEP 4

CLEAVE MASS-LABEL BY PHOTOLYSIS OR CHEMICAL CLEAVAGE AND REMOVE SUPERNATANT FOR ANALYSIS IN MASS SPECTROMETER. CLEAVAGE LEAVES 3' -OH AVAILABLE FOR FURTHER EXTENSION.

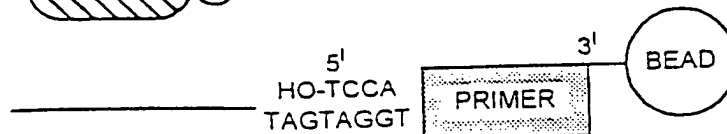
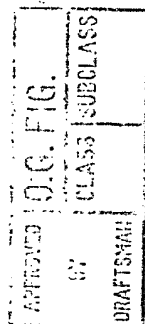


FIG. 1A



66660-TH9THE60

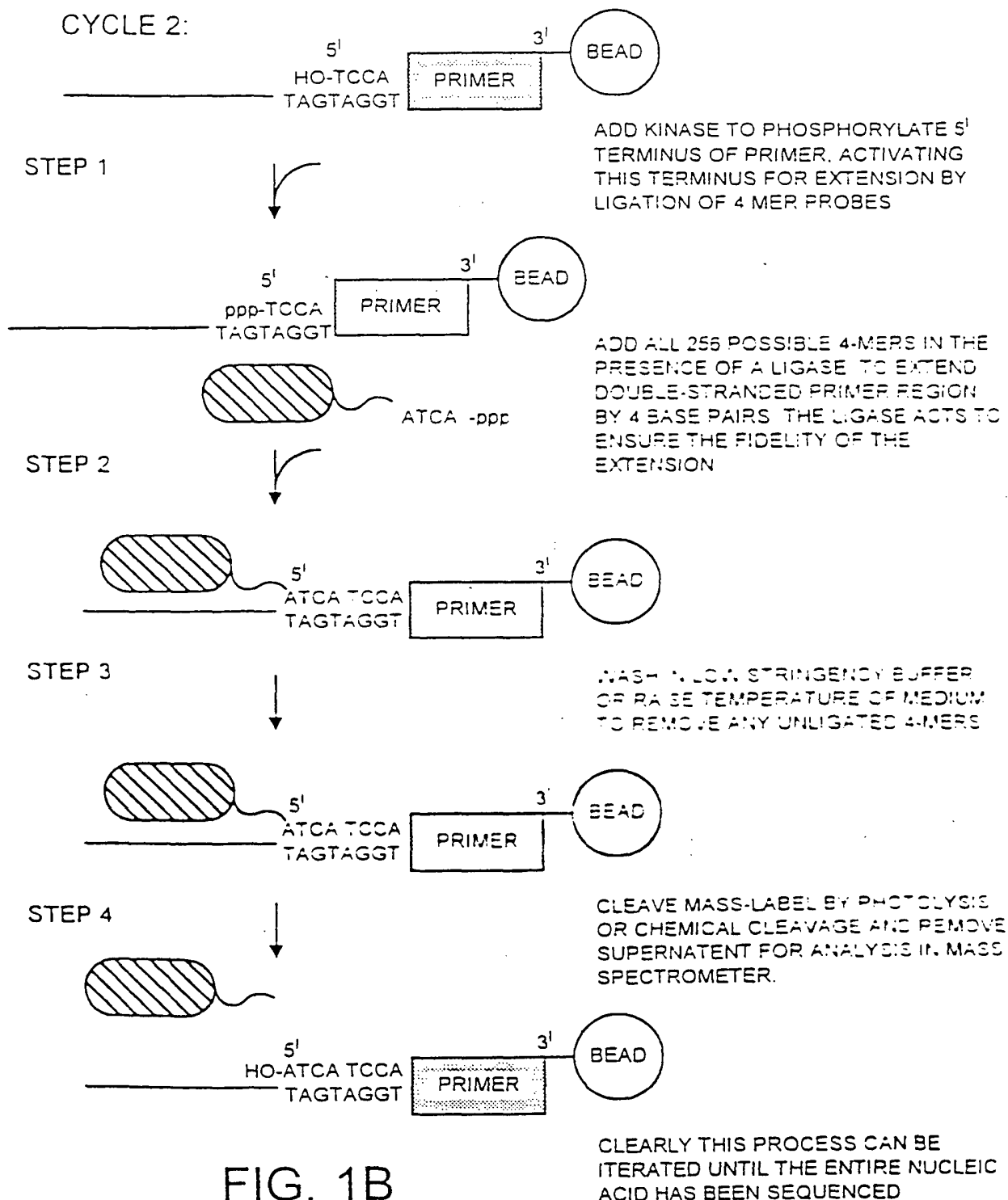
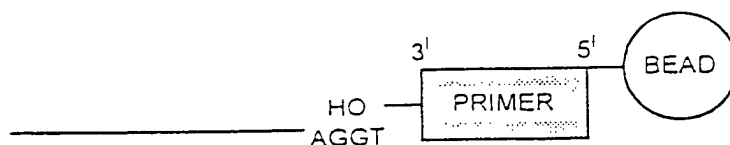
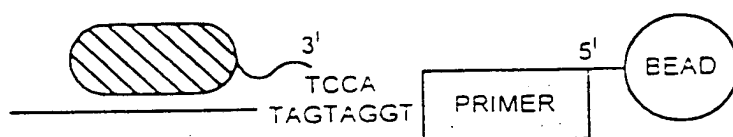


FIG. 1B

CYCLE 1:

DNA PREPARED BY VARIOUS
MEANS FROM ANY SOURCE

STEP 1

ADD ALL 256 POSSIBLE 4-MERS IN THE
PRESENCE OF A LIGASE, TO EXTEND
DOUBLE-STRANDED PRIMER REGION
BY 4 BASE PAIRS. THE LIGASE ACTS TO
ENSURE THE FIDELITY OF THE
EXTENSION

STEP 2

WASH IN LOW STRINGENCY BUFFER
OR RAISE TEMPERATURE OF MEDIUM
TO REMOVE ANY UNLIGATED 4-MERS

STEP 3

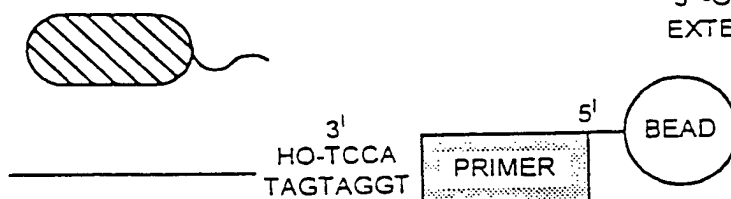
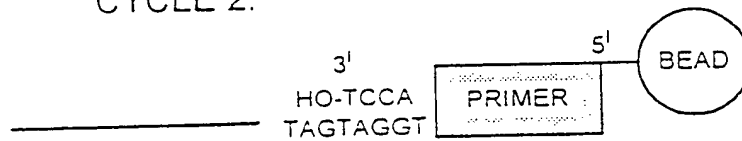
CLEAVE MASS-LABEL BY PHOTOLYSIS
OR CHEMICAL CLEAVAGE AND REMOVE
SUPERNATANT FOR ANALYSIS IN MASS
SPECTROMETER. CLEAVAGE LEAVES
3' -OH AVAILABLE FOR FURTHER
EXTENSION.

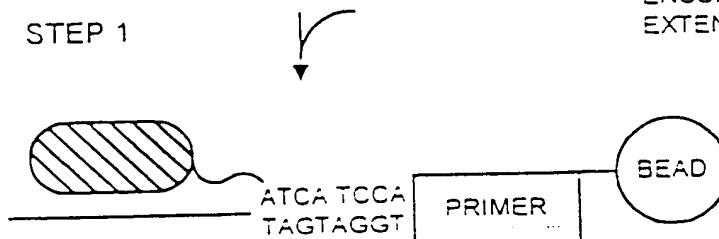
FIG. 2A

CYCLE 2:

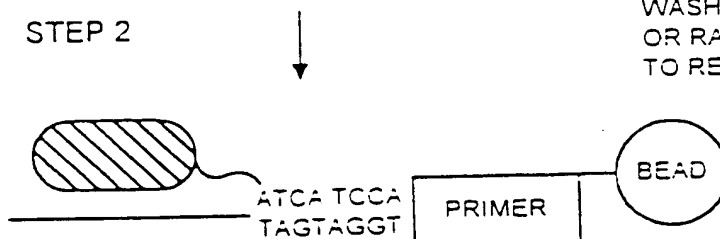


ADD ALL 256 POSSIBLE 4-MERS IN THE PRESENCE OF A LIGASE, TO EXTEND DOUBLE-STRANDED PRIMER REGION BY 4 BASE PAIRS. THE LIGASE ACTS TO ENSURE THE FIDELITY OF THE EXTENSION.

STEP 1

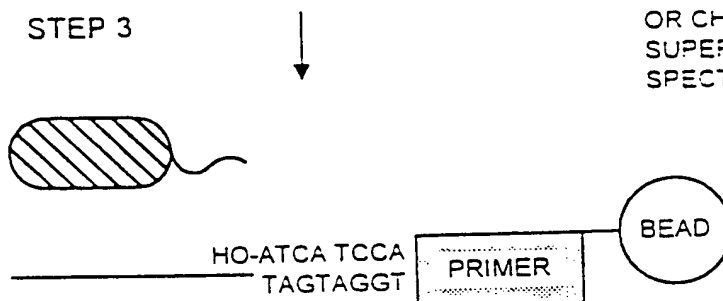


STEP 2



WASH IN LOW STRINGENCY BUFFER OR RAISE TEMPERATURE OF MEDIUM TO REMOVE ANY UNLIGATED 4-MERS.

STEP 3



CLEAVE MASS-LABEL BY PHOTOLYSIS OR CHEMICAL CLEAVAGE AND REMOVE SUPERNATANT FOR ANALYSIS IN MASS SPECTROMETER.

CLEARLY THIS PROCESS CAN BE ITERATED UNTIL THE ENTIRE NUCLEIC ACID HAS BEEN SEQUENCED.

FIG. 2B

APPROVED	O.C. FIG.
BY	CLASS / CUGGLASS
	DRAFTSMAN

66060"TH9TE60

BIOTIN — NNNGGCC
NNNCCGG
 ADAPTOR 1

BspMI RECOGNITION
 SEQUENCE
 ↘
NNNGCAGGTNNNN
NNNCGTCCANNNN
 ADAPTOR 2

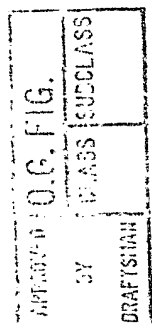
THIS ADAPTOR WOULD ALLOW
 FRAGMENTS TO BE SORTED AFTER A
 DETERMINING A TERMINUS

BsaI RECOGNITION
 SEQUENCE
 ↘
NNNGGTCTCNNNN
NNNCCAGAGNNNN
 ALTERNATIVE
 ADAPTOR 2

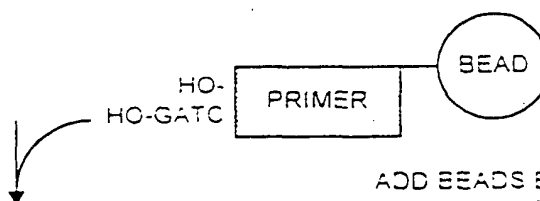
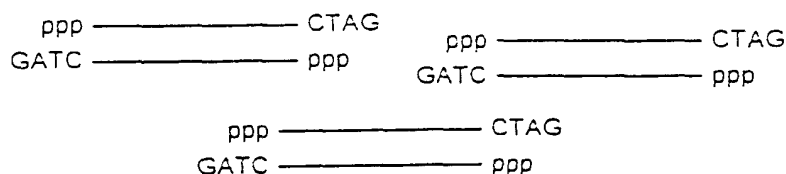
THIS ADAPTOR WOULD ALLOW
 FRAGMENTS TO BE REIMMOBILISED
 ON BEADS USING THE KNOWN BsaI
 STICKY-END THIS WOULD ALLOW
 FURTHER PROCESSING BEFORE
 BEGINNING TO SEQUENCE.

FIG. 3

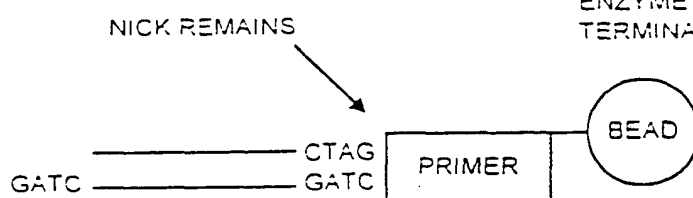
ADAPTORS TO GENERATE DISTINCT
 TERMINI IN GENERIC NUCLEIC ACIDS.



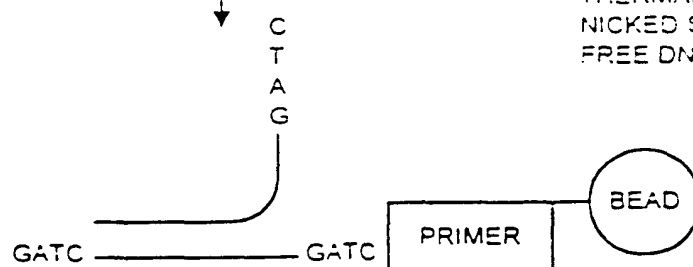
DNA CLEAVED WITH A RESTRICTION
ENZYME LIKE BamHI.



ADD BEADS BEARING A DOUBLE-STRANDED
OLIGONUCLEOTIDE WITH A STICKY-END
COMPLEMENTARY TO RESTRICTION
ENZYME STICKY-ENDS BUT WITH NO
TERMINAL PHOSPHATE GROUPS



THERMALLY DENATURE STRANDS SO
NICKED STRAND IS RELEASED WASH THE
FREE DNA AWAY



SINGLE-STRANDED PRIMED DNA REMAINS



DIAGRAM TO SHOW PREPARATION OF
SINGLE-STRANDED DNA FOR PRIMER
EXTENSION SEQUENCING.

FIG. 4

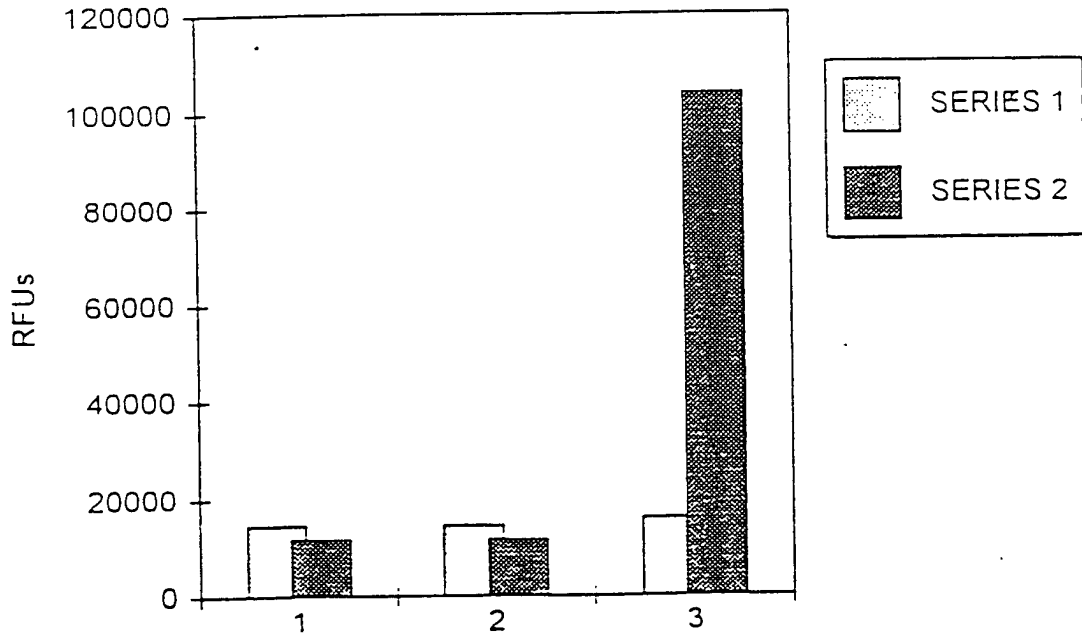


FIG. 5

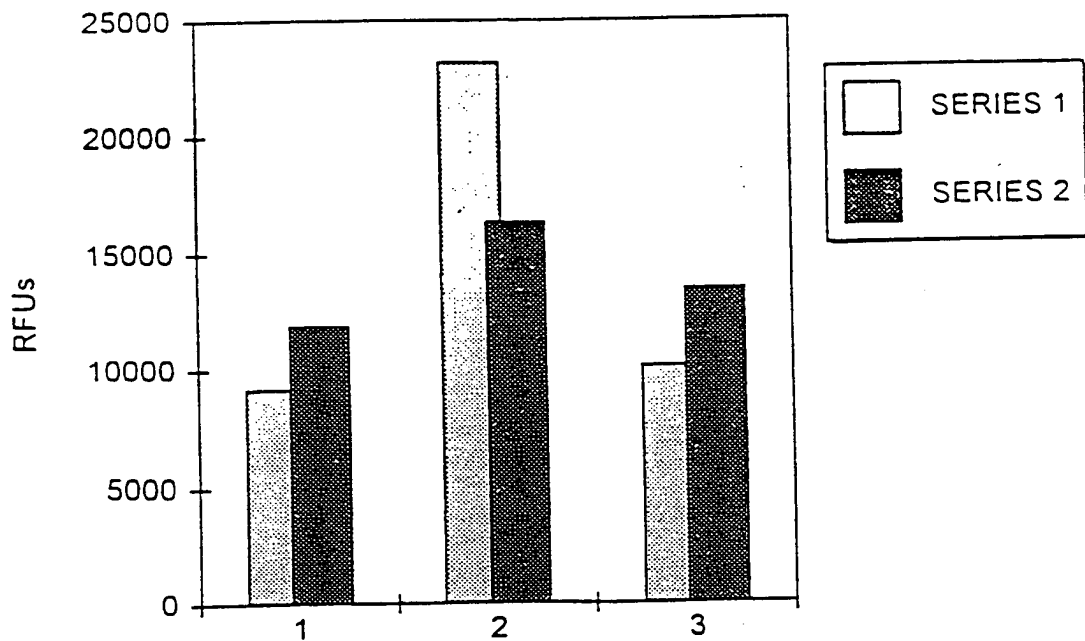


FIG. 6

APPROVED	O.G. FIG.
BY	CLASS
DRAFTSMAN	SUBCLASS

566050" T49T4E50

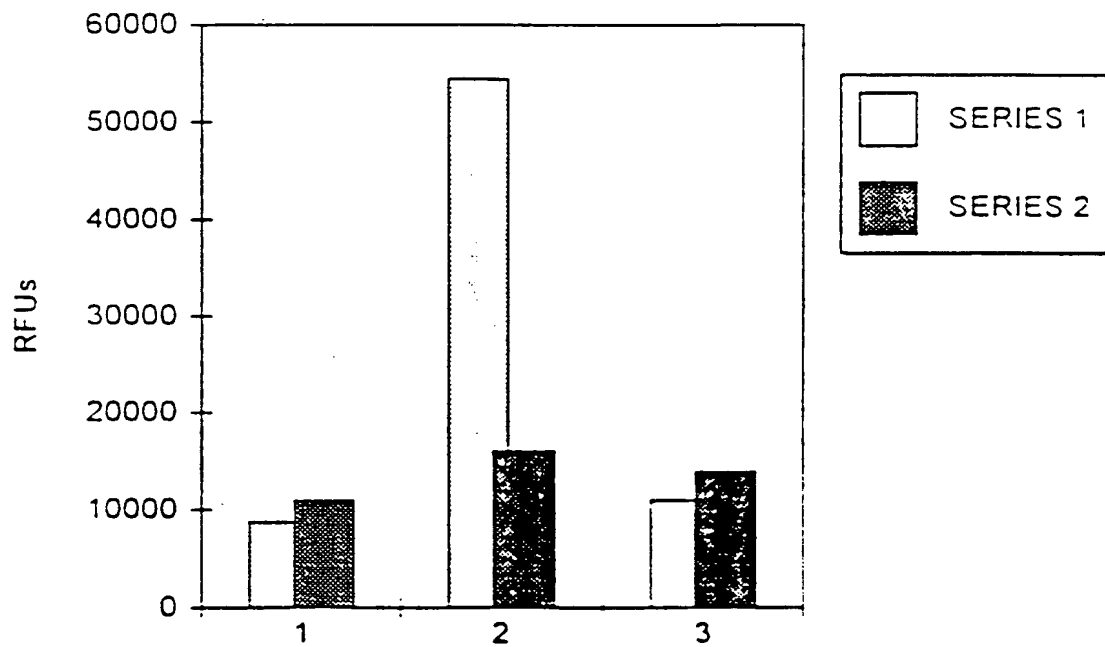


FIG. 7